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Disintegration of the *Micareaceae* (lichenized *Ascomycota*): a molecular phylogeny based on mitochondrial rDNA sequences

Heidi L. ANDERSEN and Stefan EKMAN

Department of Biology, University of Bergen, Allégaten 41, N-5007 Bergen, Norway.
E-mail: Heidi.Andersen@bot.uib.no

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The phylogeny of the family *Micareaceae* and the genus *Micarea* was studied using mitochondrial small subunit ribosomal DNA sequences. Phylogenetic reconstructions were performed using Bayesian MCMC tree sampling and a maximum likelihood approach. The *Micareaceae* in its current sense is highly heterogeneous, and *Helocarpon*, *Psilolechia*, and *Scutula*, all thought to be close relatives of *Micarea*, are shown to be only distantly related. The genus *Micarea* is paraphyletic unless the entire *Pilocarpaceae* and *Ectolechiaceae* are included, as also indicated by an expected likelihood weights test. It is suggested that the *Micareaceae* is reduced to synonymy with the *Pilocarpaceae*, which also includes the *Ectolechiaceae*, and that *Micarea* may have to be divided into a series of smaller genera in the future. *Micarea* species with a 'non-micareoid' photobiont group with *Psora* and the *Ramalinaceae*, whereas *Micarea intrusa* appears to belong in *Scoliciosporum*. Three species fall inside the paraphyletic *Micarea*: *Szczawinskia tsugae*, *Catillaria contristans*, and *Fellhaneropsis vezdae*. Tropical foliicolous taxa are nested within groups of mainly temperate and arctic-alpine distribution. A 'micareoid' photobiont appears to be plesiomorphic in the *Pilocarpaceae* but has been lost a few times.

INTRODUCTION

A recent study of the phylogenetic position of the family *Micareaceae* showed that it belongs in the *Lecanorales* close to the *Ramalinaceae* (Andersen & Ekman 2004). A close relationship between *Micarea s. str.* and members of the *Pilocarpaceae* was hypothesized. However, the study questioned the monophyly of the *Micareaceae* and the type genus *Micarea* in their current circumscriptions, mainly owing to the inclusion in the *Micareaceae* of *Psilolechia* and *Micarea* species with a 'non-micareoid' photobiont. The majority of *Micarea* species have a 'micareoid' photobiont, with small (4–7 μm diam), thin-walled, and often paired cells that become penetrated by fungal haustoria (Coppins 1983, Hedlund 1891, 1892).

The *Micareaceae*, when first informally suggested by Poelt (1974), included *Micarea*, *Roccellinastrum*, and *Scoliciosporum*. Later, when Vězda & Hafellner (in Hafellner 1984), formally described the family they included only the genera *Micarea* and *Psilolechia*. Eriksson & Hawksworth (1987) added the genus *Helocarpon* to the *Micareaceae* and re-included *Roccellinastrum* with a questionmark. Subsequently, the genus *Scutula* was added to the *Micareaceae* by Eriksson & Hawksworth (1993). In the latest version of the ascomycete system by Eriksson *et al.* (2004), the *Micareaceae* includes the genera *Helocarpon*, *Micarea*, *Psilolechia*, *Roccellinastrum*, and *Scutula*. These genera are recognised by a crustose thallus, chlorococcoid photobiont, usually immarginate and convex biatorine apothecia with a poorly developed proper exciple, simple or sparsely branched to abundantly branched and anastomosed paraphyses, asci with an apical cushion surrounded by a tube-structure, colourless and simple (ellipsoid or tear-shaped) to transversely septate ascospores lacking perispore, immersed, sessile or stalked pycnidia, and an abundance of conidial types.

The *Micareaceae* is possibly closely related to the *Pilocarpaceae* (Andersen & Ekman 2004). The *Pilocarpaceae* has many of the same features as the *Micareaceae*, but has, by tradition, been treated as separate owing to the mainly tropical instead of temperate/artic distribution, which has dictated that hardly anyone had overview and taxonomic authority over both groups. It was, however, suggested by Kalb, Lücking & Sérusiaux (2000) and Lücking (1997, 2004) that 'primitive or ancestral *Pilocarpaceae* had sessile, biatorine apothecia with prosoplectenchymatous excipulum or an excipulum composed of branched and anastomosing hyphae, such as in the related genera *Micarea* and *Mycobilimbia*' (Lücking 2004).

Table 1. List of species and specimens used for generating new mtSSU rDNA sequences, with familial affiliation according to Eriksson *et al.* (2004), Ekman (2001) and Holien & Tønsberg (2002).

Species	Family	Source[a]	GenBank accession no.
Adelolecia pilati	*Ramalinaceae*	Austria, *Ekman 3373*	AY567713
Bacidia rubella	*Ramalinaceae*	Sweden, *Ekman 3021*	AY567723
B. trachona	*Ramalinaceae*	Sweden, *Andersen 99*	AY567784
Bacidina phacodes	*Ramalinaceae*	Sweden, *Ekman 3414*	AY567725
Badimia dimidiata	*Ectolechiaceae*	Costa Rica, *Lücking 1601*	AY567774
Bapalmuia palmularis	*Pilocarpaceae*	Costa Rica, *Lücking 16003*	AY567781
Buellia disciformis	*Physciaceae*	Norway, *Tønsberg 28335a*	AY567727
Byssolecania variabilis	*Pilocarpaceae*	Costa Rica, *Lücking 16033b*	AY567780
Byssoloma leucoblepharum	*Pilocarpaceae*	Portugal, *Ekman 3502*	AY567778
B. marginatum	*Pilocarpaceae*	USA, *Tønsberg 27125*	AY567777
B. meadii	*Pilocarpaceae*	USA, *Ekman L1130* (LD)	AY567776
B. subdiscordans	*Pilocarpaceae*	USA, *Tønsberg 25968*	AY567779
Calopadia foliicola	*Ectolechiaceae*	Costa Rica, *Lücking 16011*	AY567782
Carbonea supersparsa	*Lecanoraceae*	Norway, *Ekman s.n.*	AY567773
Catillaria alba	*Catillariaceae*	Sweden, *Knutsson 2002–080* (hb Knutsson)	AY567771
C. contristans	*Catillariaceae*	Australia, *Kantvilas 466/00*	AY567757
C. erysiboides	*Catillariaceae*	Norway, *Holien 7731* (hb Holien)	AY567732
Cladonia peziziformis	*Cladoniaceae*	Norway, *Ekman 3295*	AY567716
Cliostomum corrugatum	*Ramalinaceae*	Sweden, *Ekman 3115*	AY567722
Crocynia gossypina	*Crocyniaceae*	Costa Rica, *Lücking 16052*	AY567766
Fellhanera bouteillei	*Pilocarpaceae*	Sweden, *Ekman 3417*	AY567787
F. subtilis	*Pilocarpaceae*	Germany, *Tønsberg 28199*	AY567786
F. viridisorediata	*Pilocarpaceae*	Switzerland, *Tønsberg 27375*	AY567775
Fellhaneropsis vezdae	*Pilocarpaceae*	Sweden, *Knutsson 97–229* (hb Knutsson)	AY567744
Frutidella caesioatra	*Ramalinaceae*	Norway, *Andersen 91*	AY567765
Helocarpon crassipes	*Micareaceae*	Sweden, *Kanz & Printzen 5459* (hb Printzen)	AY567728
Lasioloma arachnoideum	*Ectolechiaceae*	Costa Rica, *Lücking 16005*	AY567783
Lecania cyrtella	*Ramalinaceae*	Sweden, *Ekman 3017*	AY567720
Lecanora aff. allophana	*Lecanoraceae*	Sweden, *Ekman 3434*	AY567710
L. intumescens	*Lecanoraceae*	Norway, *Ekman 3162*	AY567715
Lecidea turgidula	*Lecideaceae*	Sweden, *Ekman 3416*	AY567788
Lecidella meiococca	*Lecanoraceae*	Sweden, *Ekman 3101*	AY567714
Micarea adnata	*Micareaceae*	Norway, *Andersen 48*	AY567751
M. alabastrites	*Micareaceae*	Norway, *Andersen 17*	AY567764
M. assimilata	*Micareaceae*	Sweden, *Kanz & Printzen 5449* (hb Printzen)	AY567739
M. bauschiana	*Micareaceae*	Norway, *Andersen 83*	AY567770
M. botryoides	*Micareaceae*	Norway, *Andersen 79b*	AY567741
M. cinerea	*Micareaceae*	Norway, *Tønsberg 28572*	AY567763
M. clavopycnidiata	*Micareaceae*	USA, *Tønsberg 27215*	AY567747
M. coppinsii	*Micareaceae*	Norway, *Tønsberg 26075*	AY567761
M. denigrata	*Micareaceae*	Sweden, *Koffman 5* (hb Koffman)	AY567759
M. elachista	*Micareaceae*	Sweden, *Koffman 399* (hb Koffman)	AY567755
M. erratica	*Micareaceae*	Sweden, *Arup 99192* (hb Arup)	AY567737
M. hedlundii	*Micareaceae*	Russia, *Hermansson 4927* (UPS)	AY567750
M. intrusa	*Micareaceae*	Norway, *Ekman s.n.*	AY567767
M. lapillicola	*Micareaceae*	Czech Republic, *Printzen s.n.* (hb Printzen)	AY567735
M. leprosula	*Micareaceae*	Norway, *Andersen 35*	AY567762
M. lignaria var. *lignaria*	*Micareaceae*	Norway, *Andersen 18*	AY567748
M. lithinella	*Micareaceae*	Norway, *Andersen 80b*	AY567734
M. lynceola	*Micareaceae*	Czech Republic, *Palice 1.X.1996* (UPS)	AY567738
M. melaena	*Micareaceae*	Norway, *Andersen 25*	AY567743
M. micrococca	*Micareaceae*	Norway, *Andersen 34*	AY567749
M. misella	*Micareaceae*	Norway, *Andersen 73*	AY567752
M. myriocarpa	*Micareaceae*	Norway, *Andersen 37*	AY567736
M. nitschkeana	*Micareaceae*	Czech Republic, *Printzen s.n.* (hb Printzen)	AY567758
M. paratropa	*Micareaceae*	Norway, *Andersen 94*	AY567740
M. peliocarpa	*Micareaceae*	Norway, *Andersen 29*	AY567760
M. prasinella	*Micareaceae*	USA, *McCune 25337*	AY567745
M. pycnidiophora	*Micareaceae*	USA, *Tønsberg 30881*	AY567754

Table 1 (*cont.*)

Species	Family	Source[a]	GenBank accession no.
M. stipitata	*Micareaceae*	USA, *Ekman s.n.*	AY567753
M. sylvicola	*Micareaceae*	Sweden, *Ekman 3629*	AY567768
M. synotheoides	*Micareaceae*	Norway, *Andersen 47*	AY567756
M. turfosa	*Micareaceae*	Norway, *Andersen 59*	AY567742
Miriquidica garovaglii	*Lecanoraceae*	Norway, *Ekman s.n.*	AY567711
Muhria urceolata	*Stereocaulaceae*	Norway, *Timdal 8612* (O)	AY567717
Myxobilimbia sabuletorum	*Ramalinaceae*	Norway, *Ekman 3091*	AY567721
Physcia adscendens	*Physciaceae*	Denmark, *Christensen & Bille L-65144*	AY567726
Protomicarea limosa	*Lecideaceae*	Norway, *Andersen 92*	AY567733
Psilolechia clavulifera	*Micareaceae*	Norway, *Andersen 128*	AY567731
P. leprosa	*Micareaceae*	Norway, *Tønsberg & Botnen 27362*	AY567730
P. lucida	*Micareaceae*	Norway, *Andersen 8*	AY567729
Psora decipiens	*Psoraceae*	Norway, *Ekman 3327*	AY567772
Pyrrhospora quernea	*Lecanoraceae*	Sweden, *Ekman 3019*	AY567712
Scoliciosporum chlorococcum	*Lecanoraceae*	Austria, *Ekman 3390*	AY567768
S. umbrinum	*Lecanoraceae*	Norway, *Ekman 3005*	AY567719
Scutula krempelhuberi	*Micareaceae*	Sweden, *Wedin 6356* (UPS)	AY567789
S. miliaris	*Micareaceae*	Sweden, *Wedin 6850* (UPS)	AY567790
Sporopodium antonianum	*Ectolechiaceae*	Costa Rica, *Lücking 16002d*	AY567785
Stereocaulon pileatum	*Stereocaulaceae*	Norway, *Tønsberg 27339*	AY567718
Szczawinskia tsugae	*Micareaceae*	USA, *Tønsberg 30044*	AY567746
Toninia cinereovirens	*Ramalinaceae*	Norway, *Haugan & Timdal 7953* (O)	AY567724

[a] The specimens are deposited in herbarium BG unless otherwise stated.

The aim of this paper is to clarify the relationships between the genera and species groups of the *Micareaceae*, thus continuing the work of Andersen & Ekman (2004) at a lower taxonomic level.

MATERIAL AND METHODS

Specimens

New sequences from the mitochondrial small subunit ribosomal DNA (mtSSU rDNA) were obtained from 81 species (Table 1). In addition to species from the *Micareaceae* in the sense of Eriksson *et al.* (2004), representatives from the *Pilocarpaceae*, *Ectolechiaceae*, *Ramalinaceae*, *Lecanoraceae*, and other families believed to be close relatives of the *Micareaceae* were included in the analysis.

DNA extraction, PCR amplification, and DNA sequencing

DNA was extracted using the DNeasy Plant Mini Kit™ (Qiagen, Hilden). PCR amplification of the mtSSU rDNA was performed with the primers mrSSU1, mrSSU2, mrSSU2R, mrSSU3R (Zoller, Scheidegger & Sperisen 1999), and MSU7 (Zhou & Stanosz 2001). The PCR mixture consisted of 1 × PCR buffer (Applied Biosystems, Foster City, CA), 1.5 mM $MgCl_2$ (Applied Biosystems), 800 μM total dNTPs (Promega, Madison, WI), 0.7 μM of each primer, 1 U of the enzyme AmpliTaq Gold DNA polymerase (Applied Biosystems), and a variable amount of extracted DNA. The PCR cycling parameters included an initial hold at 95 °C for 10 min, then denaturating at 95 ° for 60 s, annealing at 62 ° for 60 s, decreasing 1 ° per cycle for the first 6 of the 40 cycles (touchdown), and polymerisation at 72 ° for 105 s.

Direct sequencing of PCR products in both directions was performed using the PCR primers. Cycle sequencing was carried out using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems), and run on an ABI Prism 3700 DNA analyzer (Applied Biosystems). Sequences were assembled using SeqMan II, version 4.05 (DNASTAR).

Sequence alignment

Introns were excised from the sequences and removed before further analysis. The sequences were aligned using SAM (Sequences Alignment and Modeling software system) version 3.4 (Hughey, Karplus & Krogh 2003, Hughey & Krogh 1996; http://bioweb.pasteur.fr/seqanal/motif/sam-uk.html), followed by manual adjustment. Ambiguous alignment was excluded from further analyses. The alignment was deposited in TreeBASE (http://www.herbaria.harvard.edu/treebase/) under matrix accession number M2029.

Phylogenetic analyses

Phylogenetic analyses using two different likelihood approaches were carried out, Bayesian inference and maximum likelihood. *Physcia adscendens* and *Buellia disciformis* were chosen as outgroup based on the result of Andersen & Ekman (2004).

A likelihood ratio test (Huelsenbeck & Crandall 1997), as implemented in the software MODELTEST 3.06 (Posada & Crandall 1998), was performed to identify a suitable substitution model. The critical value of rejection (alpha level) was Bonferroni adjusted to 0.008 in order to maintain an overall significance level of 0.05. The general time reversible model (GTR) (Tamura & Nei 1993) was found to be optimal, including invariability (I) in a fraction of sites and substitution rate heterogeneity among nucleotide sites according to a gamma model (G).

In order to estimate the number of discrete categories to utilise in the gamma model, a neighbour-joining tree was calculated using the JC69 model of evolution. Under the GTR + I + G model, the likelihood for this tree was estimated under a varying number of gamma categories from 2–20. Each addition of a category was treated as a likelihood ratio test with df = 1 and a significance cut-off level of 0.05 in the chi-square test. The number of rate categories thereby chosen was six.

Bayesian tree inference with Markov chain Monte Carlo (MCMC) sampling was performed using MrBayes version 3.0 (Ronquist & Huelsenbeck 2003). A GTR + I + dG6 likelihood model was used. Bayesian prior distributions were set to uniform for the tree topology, the gamma shape parameter, and the proportion of invariable sites, a flat Dirichlet for the rate matrix and the state frequencies, and to an unconstrained exponential for the branch lengths. The MCMC was run using eight parallel chains incrementally heated by a temperature of 0.2, starting from a random tree. To assure that the chains had reached stationarity at the same ln likelihood, the MCMC analysis was repeated several times from different random starting trees. Every tenth tree, including branch lengths, was saved. In all, 2 M generations were sampled. To exclude a suitable number of generations as burn-in period, ln likelihood was plotted against numbers of generations. Consequently, 5000 trees from 50 000 generations were removed from the further analysis. Thus, 195 000 trees from the remaining 1 950 000 generations were used to calculate a consensus tree with all compatible groups, and posterior probabilities.

MetaPIGA 1.0.2b (Lemmon & Milinkovitch 2002a) was used to calculate alternative branch support due to uncertainties about the posterior probabilities. Several authors (i.e. Suzuki, Glazko & Nei 2002, Cummings *et al*. 2003, Douady *et al*. 2003, Erixon *et al*. 2003, Simmons, Pickett & Miya 2004) have argued that Bayesian branch support in its current implementation can be excessive compared to other measures of branch support and should preferably be used in combination with other measures; but see Alfaro, Zoller & Lutzoni (2003) and Wilcox *et al*. (2002) for an alternative view. The analysis was carried out using the metapopulation genetic algorithm (metaGA) (Lemmon & Milinkovitch 2002b) as implemented in the software MetaPIGA. The search was performed using the HKY85 likelihood model, as this is the most parameter-rich model implemented in MetaPIGA. Rate heterogeneity was estimated prior to the search, using six rate categories and with the T_i/T_v ratio optimised every 200 generations. The search was replicated 250 times, each with a noisy-neighbour-joining (NNJ) starting tree, and strict consensus pruning among four populations. Posterior branch support values were computed from the 1000 resulting trees.

A maximum likelihood (ML) analysis was carried out using PAUP* 4.0b10 (Swofford 2002). The tree search was performed using a ratchet approach in a likelihood context (Nixon 1999, Quicke, Taylor & Purvis 2001, Vos 2003). The ratchet is an approach that relies on iterative perturbations of the tree landscape to escape from local optima. The analysis was similar to that of Vos (2003), but started with a BIONJ tree (Gascuel 1997), which was branch-swapped using SPR. Characters were then reweighted using CI under parsimony, a single BIONJ jackknife tree was calculated, and branch-swapping reiterated with equal character weights under the ML criterion. We allowed the ratchet to run for five iterations. The likelihood model used was identical to the one used in the Bayesian inference.

Branch support for the ML tree was estimated using a bootstrap with 400 replicates. Likelihood model parameters were fixed at the values of the optimal tree found by the ratchet. Starting trees for the bootstrap-replicates were obtained with NJ, and these were swapped using NNI.

One phylogenetic null hypothesis was tested, namely whether the 'core' of *Micarea* found in this analysis (i.e. *Micarea* excluding *Helocarpon crassipes, M. intrusa, M. sylvicola, and M. bauschiana*, but including *Szczawinskia tsugae*, *Catillaria contristans*, and *Fellhaneropsis vezdae*), constitutes a monophyletic group. This hypothesis was tested using the expected likelihood weights (ELW) test of Strimmer & Rambaut (2002). The analysis followed that of Andersen & Ekman (2004). A thorough test requires as many 'good' trees as possible to be included, and that their weights be calculated over a reasonably large number of bootstrap replicates. In our data, this imposed an excessive computational burden. Therefore, likelihood weights were approximated here using weighted parsimony instead. Character transformation weights were calculated using the approach of Lutzoni (1997) and Ekman (2001). Violations against triangle inequality were present but adjusted by PAUP*. 1000 bootstrap replicates were generated with SEQBOOT in the PHYLIP 3.6 package (Felsenstein 2002). The tree sample included a total of 2000 unique trees, *viz*. the ML tree, the 1998 trees with the highest likelihood in the MCMC tree sample, as well as the tree agreeing best with the constraint inherent in the hypothesis. The actual calculations were performed using two Perl scripts (elw.pl and calcwts.pl) available from http://hades.biochem.dal.ca/Rogerlab/Software/software.html (Silberman *et al*. 2002).

RESULTS

The final alignment consisted of 81 taxa with 1241 characters. Primer positions and ambiguous alignments were excluded, resulting in 841 aligned positions.

A majority-rule consensus tree with all compatible groups, average branch-lengths, and posterior probabilities of branches from the Bayesian MCMC tree sample is provided in Fig. 1. This tree also includes branch support from the MetaPIGA analysis. Estimated average values ($\pm$one standard deviation) of the likelihood model parameters in the Bayesian inference were $\pi_A = 0.358 \pm 0.012$, $\pi_c = 0.114 \pm 0.008$, $\pi_G = 0.197 \pm 0.011$, $\pi_T = 0.332 \pm 0.013$, $r_{AC} = 1.594 \pm 0.296$, $r_{AG} = 7.123 \pm 0.828$, $r_{AT} = 1.878 \pm 0.244$, $r_{CG} = 0.915 \pm 0.208$, $r_{CT} = 9.649 \pm 1.460$, $p_{inv} = 0.243 \pm 0.033$, and $\alpha = 0.542 \pm 0.063$.

The ML tree including bootstrap branch support is provided in Fig. 2. The ln likelihood of the best tree found was -13812.6732, and the corresponding estimated optimal values of the model parameters were $\pi_A = 0.356$, $\pi_c = 0.125$, $\pi_G = 0.193$, $\pi_T = 0.326$, $r_{AC} = 1.286$, $r_{AG} = 6.624$, $r_{AT} = 1.818$, $r_{CG} = 0.790$, $r_{CT} = 7.968$, $p_{inv} = 0.274$, and $\alpha = 0.581$.

The two resulting trees, the Bayesian consensus and the ML tree, are very similar and the main trends are the same. The trees differ only in their weakly supported parts, for example in the internal branches of the *Lecanoraceae* and the *Ramalinaceae*. The three different types of branch support correspond closely. There is not a single instance when a branch has significant posterior probability ($\geqslant 95\%$) but low bootstrap support ($< 80\%$). However, there are five examples of the reverse, branches having high bootstrap support ($\geqslant 80\%$) but insignificant posterior probability ($< 95\%$).

The hypothesis of a modified circumscription of *Micarea* as a monophyletic group was rejected by the ELW test. The best tree agreeing with the constraint inherent in the hypothesis was outside a 99.9% confidence limit of the true tree.

DISCUSSION

The *Micareaceae* in the sense of Eriksson *et al*. (2004) included five genera: *Micarea*, *Helocarpon*, *Psilolechia*, *Scutula*, and *Roccellinastrum*. The Bayesian consensus tree and the ML tree resulting from the present analyses (Figs 1–2) confirm that the *Micareaceae* in that circumscription is an artificial family, as also indicated by Andersen & Ekman (2004). The species of *Micarea* with a 'micareoid' photobiont, to which the type species, *M. prasina*, of *Micarea* as well as the *Micareaceae* belongs, form a well-supported monophyletic group together with the *Pilocarpaceae* and *Ectolechiaceae*. However, none of these groups appear to be monophyletic, and as a consequence, the families have to be united. Lücking, Lumbsch & Elix (1994) were the first to question the distinction between the *Ectolechiaceae* and the *Pilocarpaceae*. Subsequently, Lücking (2004) synonymized the *Ectolechiaceae* with the *Pilocarpaceae* based on morphological characters, a conclusion which is supported here. The *Pilocarpaceae* and *Ectolechiaceae* were described simultaneously by Zahlbruckner (1905), and the *Micareaceae* by Vězda & Hafellner (in Hafellner 1984). Consequently, the correct name of the family appears to be *Pilocarpaceae*, with *Micareaceae* as a synonym along with *Ectolechiaceae*. Our results do not support a close relationship between other genera suggested to belong to the '*Micareaceae*' (*Helocarpon*, *Psilolechia*, and *Scutula*) and the *Pilocarpaceae* in its expanded sense. Coppins (1983, 1992) included *Helocarpon* in *Micarea* whereas Hafellner (1984) described a new monotypic family, *Helocarpaceae*. Eriksson *et al*. (2004), on the other hand, took the middle way and accepted *Helocarpon* as a genus in the *Micareaceae*. Our results show that *H. crassipes* is not closely related to *Micarea*, or to any of the other genera classified in the *Micareaceae*. Furthermore, there is no evidence to support or reject Hafellner's *Helocarpaceae* because of weak taxon sampling of this part of the *Lecanorales*. For that reason, the closest relatives of *Helocarpon* remain unknown. The three species of *Psilolechia* included in the present analyses together form a strongly supported monophyletic group. In the Bayesian consensus tree (Fig. 1) and the ML tree (Fig. 2), the placement of *Psilolechia* is outside the *Pilocarpaceae*. However, there is no significant branch support, neither in the Bayesian inference nor in the ML bootstrap, for an alternative placement. *Scutula*, on the other hand, represented here by *S. krempelhuberi* and the type species *S. miliaris*, appears close to *Bacidina* and *Toninia* with high Bayesian posterior probabilities and ML bootstrap support (Figs 1–2). Morphological characters correspond well with the main features of the *Ramalinaceae* (in the sense of Eriksson *et al*. 2004), as discussed by Triebel, Wedin & Rambold (1997). However, reports of possibly micareoid photobiont in the two lichenized species of *Scutula*, *S. dedicata* and *S. heeri*, need confirmation. A fourth genus, *Roccellinastrum*, has been referred to the *Micareaceae* but was not included in this analysis because fresh material for DNA extraction could not be obtained.

A number of species and species-groups have been referred to *Micarea* without having any close relationship with this genus or the *Pilocarpaceae*. *Micarea sylvicola*, *M. bauschiana*, *M. tuberculata*, and *M. lutulata* form a group characterized by a 'non-micareoid' photobiont, which is an ordinary single chlorococcoid algae of larger size than the 'micareoid'. They are represented here by the two first species, and form a highly supported group together with *Psora decipiens*, and seem to be near to the *Psoraceae*. *Micarea intrusa* (Coppins 1983), known also as *Carbonea intrusa* (Aptroot *et al*. 1997) has been placed in a number of different genera (e.g. *Lecidea*, *Catillaria*, *Micarea*, and *Carbonea*), but never in *Scoliciosporum*, although

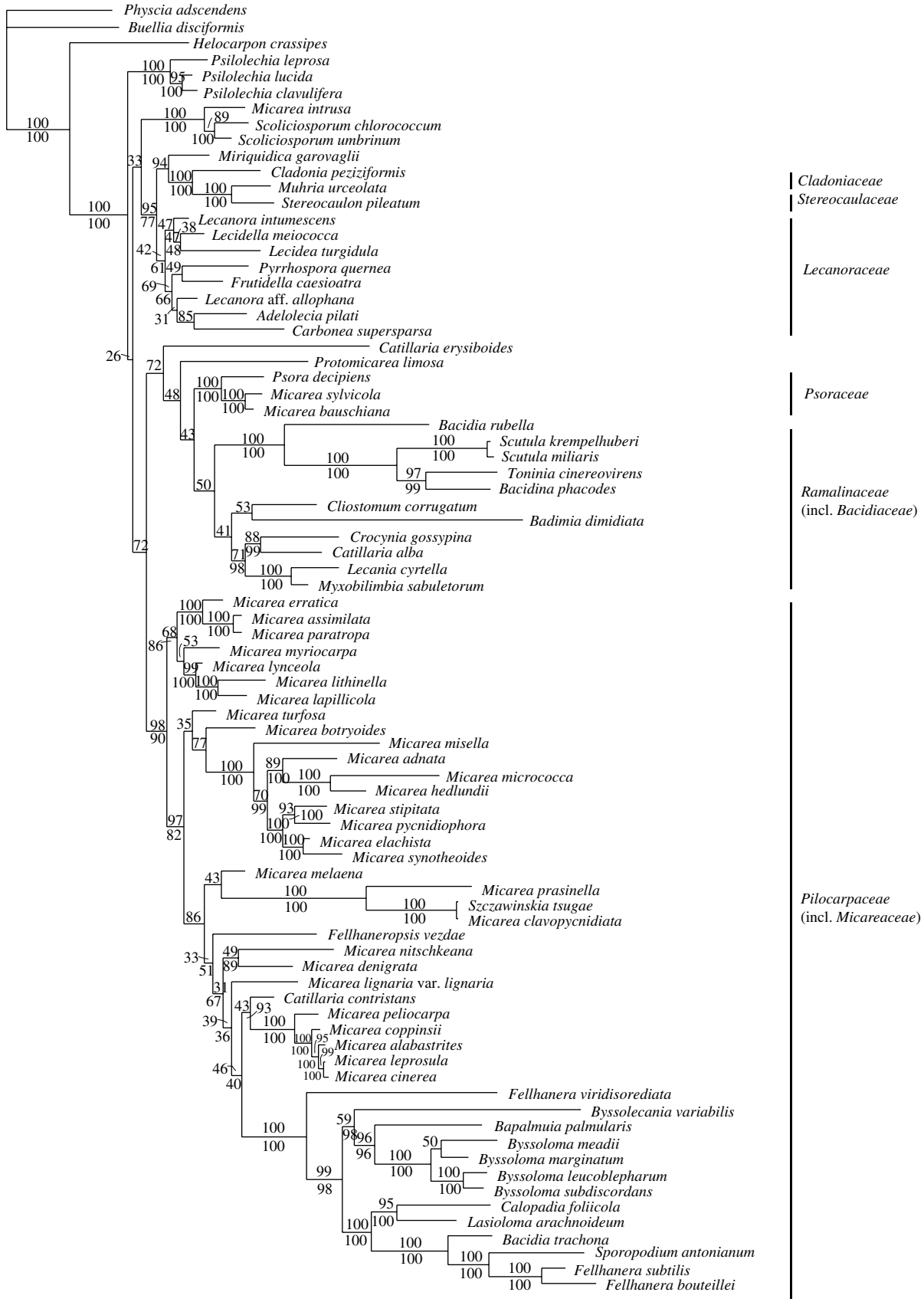

Fig. 1. 50 % majority rule consensus tree with all compatible groups and with average branch lengths, based on 195 000 trees from Bayesian MCMC tree sampling. Branch support is displayed at nodes, and consists of Bayesian posterior probabilities (above branches) and quasi-bayesian support obtained with MetaPIGA (in bold, below branches). Note that in this tree, the *Ramalinaceae* in the sense of Ekman (2001) forms an unsupported monophyletic group, in contrast to Fig. 2.

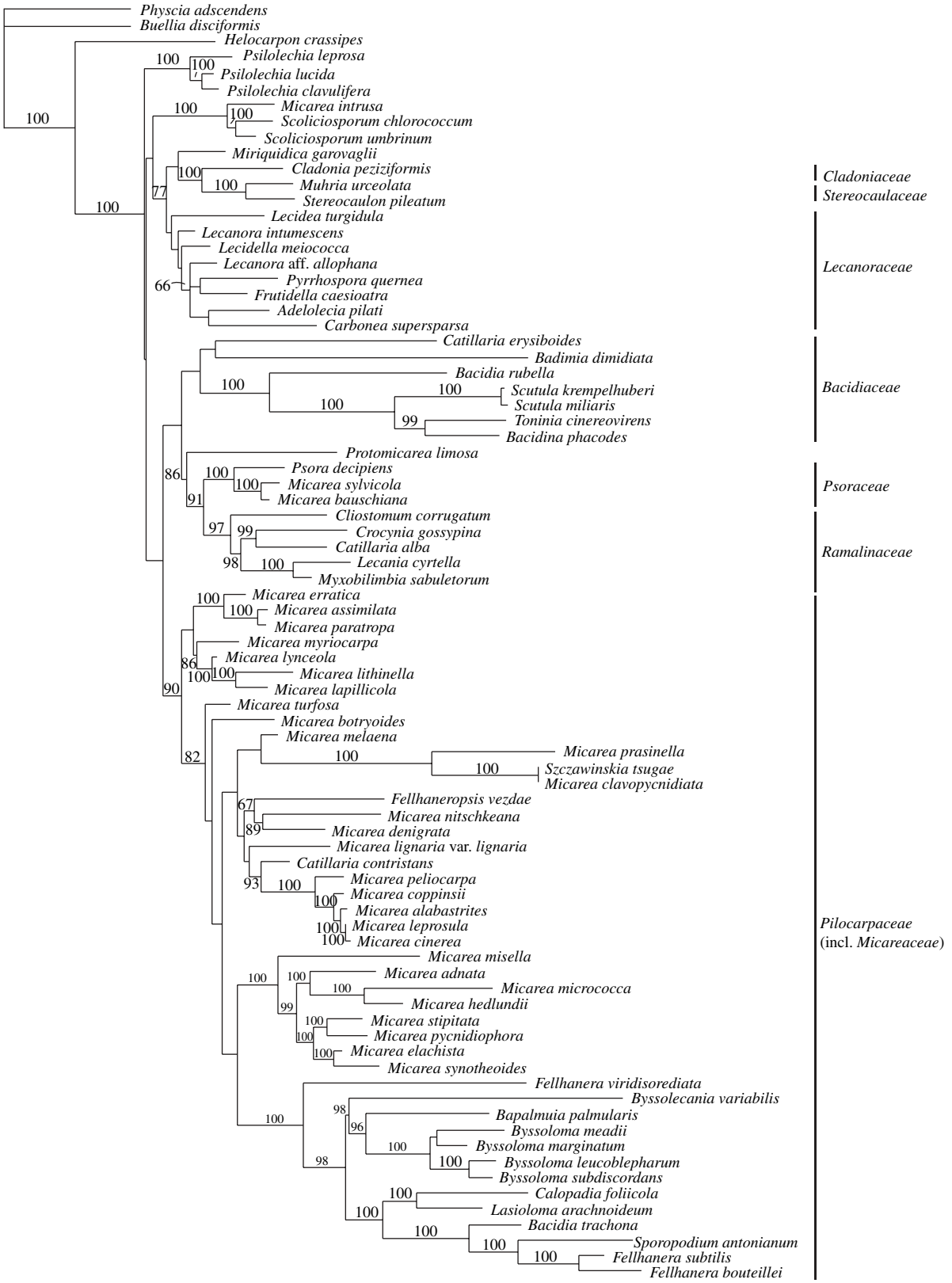

Fig. 2. The most likely tree under the GTR + I + G model with ln likelihood = −8146.61994. Values at branches are bootstrap branch support above 50 %. In this tree, the *Ramalinaceae* in the sense of Ekman (2001) is non-monophyletic unless the *Psoraceae* is included, and therefore divided into *Ramalinaceae s. str.* and *Bacidiaceae*.

Coppins (1983) mentioned the similarities with that genus. In the present study, *M. intrusa* forms a strongly supported monophyletic group with *Scoliciosporum*. *M. intrusa* does not have a 'micareoid' photobiont, but appears to have the same alga as *S. umbrinum* (Coppins 1983). In addition, the morphological features of *M. intrusa* are similar to *Scoliciosporum*. However, the phylogenetic position of *Scoliciosporum* is uncertain due to weakly supported branches and sparse sampling of this part of the tree. *Scoliciosporum* has been suggested to be a close relative of *Micarea* (Poelt 1974), which is not supported by this study, nor by Andersen & Ekman (2004). *Badimia* has been placed in the *Ectolechiaceae* (Eriksson *et al*. 2004), but in our study the type species, *B. dimidiata*, appears on a long branch inside the *Ramalinaceae* (incl. *Bacidiaceae*); (Fig. 1) or in a group containing a mixture of *Ramalinaceae* and *Psoraceae* (Fig. 2). However, resolution in this part of the trees is inadequate owing to poor branch support and it cannot at this point be concluded whether *Badimia* should be referred to the *Ramalinaceae* or *Psoraceae* (if any), if the two families should be united, or if the *Ramalinaceae* should be split into two families (*Ramalinaceae* and *Bacidiaceae*). It is suggestive that Lücking, Lumbsch & Elix (1994) provided drawings of the ascus amyloid reaction in three species of *Badimia*, which correspond closely to the ascus seen in *Psora* and relatives (Hafellner 1984). Finally, *Crocynia* was included in this study because of reported similarities in ascus amyloid reaction with *Micarea*. *Crocynia* was treated in its own family by Hafellner (1984). In our investigation, maximum likelihood bootstrap branch support (Fig. 2) as well as quasi-Bayesian MetaPIGA branch support (Fig. 1) is high for a close relationship between *Crocynia* and *Myxobilimbia*, *Lecania*, and *Catillaria alba*. However, Bayesian posterior probabilities (Fig. 1) provide no significant support for an inclusion in any group. Apparently, the position of *Crocynia* is in need of further study.

A further conclusion from this study is that *Micarea* is paraphyletic even if unrelated species and species-groups are excluded, as indicated by the phylogenetic trees (Figs 1–2) and the rejection of the null hypothesis of *Micarea* monophyly. *Micarea* may have to be divided into a series of smaller genera, although taxon sampling, gene sampling, and current branch support is yet too weak to show exactly how this could be solved taxonomically. However, there is a number of highly supported monophyletic species groups that can be identified within an expanded *Pilocarpaceae*. The group including *Micarea micrococca*, *M. hedlundii*, *M. adnata*, *M. pycnidiophora*, *M. stipitata*, *M. elachista*, *M. synotheoides*, and *M. misella* represent *Micarea* in the strict sense, as *M. micrococca* is very closely related to the type species, *M. prasina* (which could not be included in the analyses because of technical difficulties). This group is recognized by apothecia that range from mostly pallid to brown or black in *M. misella*, simple or 1-septate ascospores, and mostly by the presence of both micro- and mesoconidia. Another strongly supported group consists of *M. peliocarpa*, *M. coppinsii*, *M. alabastrites*, *M. leprosula*, and *M. cinerea*. These species all have pallid or bluish apothecia with mostly 3-septate ascospores. Their pycnidia are of two types, large and immersed, or small and sessile, with both micro- and macroconidia. This group is also recognized by the presence of gyrophoric acid. Near the base of the *Pilocarpaceae*, there are two well-supported groups that are possibly closely related. One group includes *M. erratica*, *M. assimilata*, *M. paratropa*, the second *M. lynceola*, *M. lithinella*, *M. lapillicola*, and possibly *M. myriocarpa*. Both groups have dark apothecia with simple ascospores, small, immersed pycnidia with micro and/or mesoconidia, and no secondary chemistry. *Micarea prasinella* and *M. clavopycnidiata* form a well supported group together with *Szczawinskia tsugae*, the implications of which are discussed further below. The 'traditional' *Pilocarpaceae* and *Ectolechiaceae*, both dominated by foliicolous taxa, together form a highly supported group within which there are numerous additional branches with high support. An interesting result is that there is strong support for *Fellhanera* being heterogeneous in its current circumscription, as also indicated by Lücking (2004). *Fellhanera subtilis* and *F. bouteillei* are closely related, and situated in the '*Ectolechiaceae*' branch of the *Pilocarpaceae*, close to *Sporopodium*.

A number of taxa have been shown to be unrelated to *Micarea* or any part of the *Pilocarpaceae*. However, there are also examples of the opposite situation, species or species groups with close affinities to either any part of *Micarea* or to other parts of the *Pilocarpaceae*. This concerns *Szczawinskia tsugae*, *Catillaria contristans*, *Fellhaneropsis vezdae*, and *Bacidia trachona* (Figs 1–2). *Szczawinskia* was described by Funk (1983), and proposed to belong to the *Micareaceae* by Holien & Tønsberg (2002). In the present analyses, *Szczawinskia* is nested within a non-monophyletic *Micarea*. *Szczawinskia tsugae* together with *M. clavopycnidiata* and *M. prasinella* form a very strongly supported monophyletic group. Although clearly closely related to many species traditionally included in *Micarea*, the paraphyly of *Micarea* results in the distinct possibility that the generic name *Szczawinskia* should be used for a group of species, the delimitation of which remains unclear. *Catillaria contristans* appears within the paraphyletic *Micarea* in the *Pilocarpaceae*; *C. contristans* is morphologically similar to *M. lignaria*. The genus *Fellhaneropsis* (Sérusiaux 1996) was described to accommodate the type species *F. myrtillicola* and *F. vezdae* (both formerly treated in *Bacidia*), and was thought to be closely related to the genus *Fellhanera*. However, *Fellhaneropsis vezdae* also resembles *Micarea* in many ways, especially in the apothecia, as pointed out by Lücking (2004). In our study, *Fellhaneropsis vezdae* is situated within a paraphyletic *Micarea*, but further details about its affinities remain unclear owing to poor branch support. There is no support for the

hypothesis of a close relationship with *Fellhanera*. *Bacidia trachona* clearly belongs in the *Pilocarpaceae*, more specifically in the part of the family that is dominated by foliicolous taxa. Ekman (1996) suggested that *B. trachona* should be excluded from *Bacidia s. str.* based on morphology. It should be pointed out that the name *B. trachona* has been widely misused for taxa more closely related to *Bacidia* and *Toninia*.

The phylogenetic trees reveal evolutionary trends in ecology and distribution patterns within the expanded *Pilocarpaceae* (Figs 1–2). Basal taxa are mainly arctic-alpine or cold-temperate and inhabit rock, soil, or detritus. Species of the temperate zone, occurring on bark of conifers and broad-leaved trees (mainly in Europe) or on wood (more or less world-wide) are nested inside the arctic-alpine taxa. Finally, the mainly tropical and foliicolous group (the 'traditional' *Pilocarpaceae* and *Ectolechiaceae*) appears to have evolved from a temperate ancestor, although within the tropical group a few taxa have returned to a temperate climate. Furthermore, there is a less clear and less well understood evolutionary trend in photobiont. Basal taxa (the 'traditional' *Micarea*) seem to have a 'micareoid' photobiont, but this was lost at least in *Catillaria contristans* and in the foliicolous taxa. However, further studies of the photobiont in the *Pilocarpaceae* are needed.

The present study of the '*Micareaceae*' has shown that this family is artificial, and that a number of taxa classified in the *Micareaceae* either belong in other families of the *Lecanorales* or have inconclusive relationships. *Micarea* itself, even when distantly related species are excluded, is paraphyletic and in need of being divided into an unknown number of smaller genera, which should be included in an expanded concept of *Pilocarpaceae*. In order to better understand the relationship between the species groups traditionally included in *Micarea*, more work, including more taxa and genes, is underway.

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Corresponding Editor: H. T. Lumbsch